View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by University of Southern Queensland ePrints

GLYPHOSATE IN WATERS AND SOILS FROM GENETICALLY MODIFIED CANOLA CULTIVATION IN PARKES, NSW, AUSTRALIA

Kamrun Nahar[1], Robert K. Niven[1*], Stuart Pearson[2], Kate Badek[2], Hans Riesen[2] and A.F. Mokhlesur Rahman[2]

[1]*School of Engineering and Information Technology, The University of New South Wales at ADFA, Northcott Drive, Canberra ACT 2600 Australia.*
[2]*School of Physical, Environmental and Mathematical Sciences, The University of New South Wales at ADFA, Northcott Drive, Canberra ACT 2600 Australia.*
[*]*Corresponding author. Email: r.niven@adfa.edu.au*

ABSTRACT

Investigations were conducted of farmland from the Parkes region of New South Wales, Australia, cultivated with genetically modified canola, involving the determination of glyphosate (N-(phosphonomethyl)glycine) concentrations in water and soils, and its sorption. The soils are classified as loam under the USDA system (clay 13.8-15.8%, silt 39-43%, sand 41.2-47.2%). Firstly, a low-cost fluorometric method was developed for the analysis of glyphosate in waters and soils, calibrated against analytical standards and spectrophotometric and enzyme-linked immunosorbent assay (ELISA) methods. Soil and water samples were then collected using the NEPM sampling protocol into glass containers, chilled and analysed within two weeks. The samples were collected in multiple episodes, taking account of glyphosate and pesticide crop applications. The soil and water physical and chemical properties were characterised, and glyphosate levels were determined. Field concentrations of glyphosate ranged between 0.01 - 0.067 mg/L in water and 0.10 - 0.575 mg/kg in soil. The aqueous levels lie below Australian and international drinking water guidelines, but reach a Canadian freshwater guideline. Glyphosate levels varied with time of application and rainfall events. Glyphosate sorption isotherms were also constructed by batch tests on several soils, and were fitted with Freundlich and Langmuir isotherms. Desorption tests indicated 25% to 58% of soil glyphosate is extractable by 0.1M KH_2PO_4.

Keywords: Glyphosate, Water, Soil, Fluorometric, Spectrophotometric, ELISA, Sorption Isotherm

INTRODUCTION AND BACKGROUND

Genetically modified (GM) foods have been commercially available since 2000 in the Australian market, when soybean, canola, corn and cotton were approved by Food Standards Australia and New Zealand [1] . GM canola was first grown commercially in New South Wales in 2008 by 108 growers, and that year around 9600 hectares of GM canola were planted in NSW and Victoria [2]. In 2009, the uptake of GM canola in NSW increased four-fold and over 41,000 hectares were planted [2]. Most of these areas in NSW are planted with Roundup Ready canola. 93% of GM canola growers rated the weed control achieved in their Roundup Ready canola as excellent compared to much lower ratings for the Clearfield®, Triazine tolerant and conventional canola systems [3].

Glyphosate (N-(phosphonomethyl) glycine), a white and odourless crystalline solid, is widely used as a broad-spectrum, non-selective, post-emergence herbicide to control a wide range of weeds. It is the active ingredient in several commercial products: the most well known is Roundup which is widely applied in the GM canola agriculture practice. Low-cost generic formulations, manufactured in China, are also widely available. Glyphosate is commonly used in salt form, most typically as the isopropyl amine salt. Commercial manufacturers often add additional components to create products that are convenient to handle, mix well with other agricultural products, or facilitate movement of the active ingredient into plants [4].

Glyphosate is a small molecule which has three polar functional groups (carboxyl, amino and phosphonate groups). It is strongly sorbed by soil minerals after application [5-8] [9-11], having a usual half life of 3-174 days on soil minerals and 5-91 days in water [12]. Sorption of glyphosate to soil usually occurs through the phosphonic acid group (organic compounds containing C-$PO(OH)_2$ or C-$PO(OR)_2$) in its phosphonate anion form, similarly to phosphate (PO_4^{3-}) in soil [5, 7, 13-15], even though the carboxylic group can also participate. Glyphosate soil sorption has also been interpreted by ion exchange and hydrogen bonding [16]. Previous studies in the literature suggest that the degree of sorption of glyphosate by soils or clays is a function of the cation exchange capacity (CEC), clay content [17], and concentrations of organic matter, iron and aluminium amorphous oxides [18]. It has been reported that glyphosate forms mono- and divalent anions which have a high affinity, in particular, for trivalent cations such as Al^{3+} and Fe^{3+} within the pH range 4-8 [19, 20]. As will be shown, the sorption characteristics of glyphosate explain its mobility in soil. Microbial biodegradation of glyphosate also occurs in soil, aquatic sediments and water, mainly by splitting of the C–N bond to produce

aminomethylphosphonic acid (AMPA), its principal degradation product [21].

Owing to the widespread adoption of glyphosate in Australia over the last few years, as well as the Australian Government's plans to increase cultivation of GM crops [22], there has been considerable controversy concerning possible impacts on the environment. An Australian health-based guideline was first scheduled for glyphosate in the 6th edition of the Australian Drinking Water Guidelines, of 1 mg/L glyphosate in drinking water [23]. The World Health Organization also calculated a human health-based criterion of 0.9 mg/L [21], although they did not establish a formal guideline [24]. The U.S. Environmental Protection Agency lists a Maximum Contaminant Level of 0.7 mg/L in its National Drinking Water Standards [25], and a Reference Dose of 2 mg/L (the amount which a person can consume each day over a lifetime without incurring 'appreciable risk' of negative effects) [26]. The Canadian Government has established a health-based Maximum Acceptable Concentration of 0.28 mg/L [27], also equal to the Livestock Water Guideline under the Canadian Water Quality Guidelines for the Protection of Agricultural Water Uses [28], as well as a Fresh Water Guideline of 0.065 mg/L under the Canadian Water Quality Guidelines for the Protection of Aquatic Life [29].

AIMS

The aims of this study were threefold:

1) To establish a low-cost analytical method (direct fluorometric) for the determination of glyphosate in soils and/or waters, to avoid the need for prohibitively expensive analytical methods such as High Performance Liquid Chromatography (HPLC). The method was calibrated against and used in conjunction with a commercially available enzyme-linked immunosorbent assay (ELISA) method, for the analysis of glyphosate in waters.
2) To conduct a case study investigation of glyphosate concentrations in soil and waters from an agricultural area at Parkes, NSW, used for GM canola cultivation for the last 8 years. To date, several previous farm-level case studies have been conducted worldwide [30-32]. Recently few case studies been conducted in different parts of NSW [33], but none from the Parkes region. The glyphosate sorption and desorption characteristics of soils from the case study site were also examined and well compared with another established spectrophotometric method [34].
3) To assess the human health and environmental impact of glyphosate due to GM cultivation at the case study site.

ESTABLISHMENT OF ANALYTICAL METHODS

Background

The cost of analysis of organic contaminants in environmental samples can be very expensive: for example, quoted costs for laboratory analysis of glyphosate can exceed $150 for each water sample and $300 for each soil sample. This cost can impose major constraints on the design of monitoring studies, especially in the university and agricultural sectors [35]. In the last few years the development of analytical techniques for glyphosate quantification has increased and several alternative methods have been proposed [32, 36-39]. Most methods reported in the literature for quantification of glyphosate in water, soils, fruits, crops, vegetables and other samples are based on chromatographic separation and determination [40]. Depending on the detection technique, glyphosate determination usually needs a derivatisation step; for example, analysis by gas chromatography is performed after derivatisation of glyphosate into volatile and thermally stable derivative [41, 42]. UV–visible and fluorescence detection methods are normally used for glyphosate derivatives in liquid chromatographic method [43-45]. For glyphosate quantification by LC-MS, a quite complicated derivatisation step has to be conducted to improve the chromatographic performance [46, 47]. Sorption of glyphosate on soils using ^{14}C-labelled glyphosate can also be performed in laboratory experiments [48], allowing quantification of glyphosate at low concentrations, but requires special expensive equipment for detecting ^{14}C labelled molecules.

The above analytical methods for glyphosate quantification in natural samples are not only expensive but can involve complicated, time consuming procedures. It would therefore be useful to have a simpler and faster method which is also of lower cost analysis than the above-mentioned chromatographic methods.

Since glyphosate does not contain chromophore or fluorophore groups, direct spectrophotometric and fluorometric methods can be used for its determination. Indeed, some previous studies have examined glyphosate quantification by UV-visible spectroscopy [34, 49, 50]. To the authors' knowledge, a direct fluorometric method for determination of glyphosate has not yet been developed. A simple, fast and low cost fluorometric method was therefore developed for this study and is reported herein. This method was then used

for the determination of glyphosate herbicide in environmental samples and for the construction of sorption isotherms in soils. The method is based on a glyphosate quantification by direct fluorescence detection, which involves sample extraction, derivatisation of glyphosate using 9-fluorenyl methoxycarbonyl chloride (FMOC-Cl), and measurement of emissions at 320 nm. The method is calibrated against analytical standards and a commercially available enzyme-linked immunosorbent assay (ELISA) method for the analysis of glyphosate in waters. A reported low-cost spectrophotometric method [34] was also developed in this study for the determination of glyphosate soil sorption and desorption results, which involve much higher concentrations.

Fluorometric method

Reagents

Glyphosate (PESTANAL, analytical standard) and the derivatisation reagent, 9-fluorenyl methoxycarbonyl chloride (FMOC-Cl), were obtained from Fluka (Germany). HPLC grade acetonitrile and diethyl ether were purchased from Sigma-Aldrich Australia. All other chemicals including KCl, HCl, KOH, NaOH, disodium tetraborate decahydrate ($Na_2B_4O_7.10H_2O$), potassium phosphate monobasic (KH_2P0_4) and diethylether were analytical grade.

Solution

Glyphosate standard solutions of various concentration (0, 0.01, 0.02, 0.05, 0.06, 0.09, 0.18, 0.2, 0.4, 0.5, 1, 2, 2.5, 5, 10, 12, 15, 20, 22, 25 mgL^{-1}) were prepared with analytical-standard glyphosate (PESTANAL, 99.729%) using distilled water. FMOC-Cl solutions of 1 g L^{-1} were prepared by dissolving the reagent in acetonitrile and were always prepared just before the experiments. Buffer solution (pH =9) was prepared by dissolving 15.255 g of $Na_2B_4O_7.10H_2O$ in 1000 mL of distilled water. 0.1 M EDTA solution was used for pre-treatment of samples to correct the sensitivity of the fluorogenic reagent to divalent ions in the amino-acid coupling [51].

Procedure

The quantification of glyphosate in water was performed on samples filtered through a 0.45 µm membrane. For the soil samples, glyphosate was determined after extraction of 10 g of each sample with 0.1 M KH_2PO_4; agitation (15 min); centrifugation (4000 rpm; 17 min) and filtration through Whatman no.1 filter paper. The extraction was repeated three times on solid residue to obtain a 25 mL extract from each sample, and the extracts filtered (0.45 µm membrane). The derivatisation procedure performed as: 0.5 mL of borate buffer and 0.5 mL of FMOC-Cl in acetronitrile were added to 3 mL of each sample (water sample or extract of soil sample). The mixture was then shaken in a mechanical shaker for 2 hours at room temperature, and the resulting solution mixed with 4 mL of diethyl-ether, shaken, and centrifuged at 4000 rpm for 15 min to separate diethyl-ether from the sample. Different solvents were tested for their ability to remove excess reagent (FMOC-Cl) from the solution, which otherwise would have interfered with the fluorometric measurements. Diethylether gave the best results for efficiency and decantation facilities than CH_2Cl_2 [34] and ethylacetate [52]. The extraction of the FMOC-Cl with diethylether was performed six times to get reliable results without the interference of organic matter present in the samples. The aqueous phase, which contained the derivatisation product, was then extracted and quantified by fluorometer with a fluorescence quartz cuvette. The emission acquisition was recorded at 290-450 nm wavelength range, from which the emission at 320 nm was used for glyphosate determination. All the results were quantified after the subtraction from blank correction. The blank solution was prepared by treating 3 mL of distilled water with 0.5 mL of borate buffer, 0.5 mL of FMOC-Cl and 4 mL of diethylether, shaken and centrifuged as described above.

Results

Glyphosate has three chemical groups: phosphonate, amine and carboxylate. The amine group reacts with FMOC-Cl in acetonitrile at pH 9 which gives the derivatized glyphosate as reaction product (Fig. 1.). Several emission spectra obtained from the derivatized glyphosate are shown in Fig. 2 (a). The derivatized glyphosate has an maximum emission (cps) at 320 nm. By plotting this emission as a function of glyphosate concentration a linear calibration curve (Fig.2(b)) was obtained. Reliable results were obtained (Table 1) in comparison with ELISA and spectrophotometric method with this calibration curve over the range 0.01 mg L^{-1} and 25 mg L^{-1}

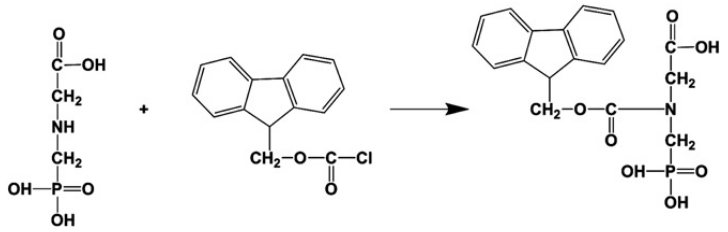
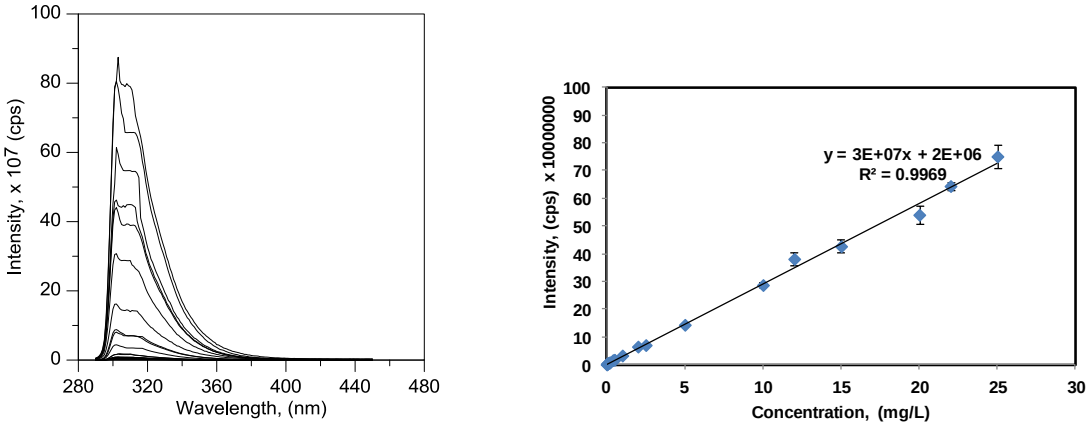


Fig.1. The derivatisation reaction scheme for the fluorometric analytical method [46].



(a) **(b)**

Fig. 2. (a) Fluorescent emission spectra of derivatised glyphosate at different concentrations, and (b) constructed calibration curve at 320 nm.

Table 1. Calibration of different methods for detection of glyphosate in waters (nd = not detected)

ELISA			Fluorometer		Spectrophotometer	
Added concentration, mg/L	Measured concentration, mg/L	% Recovery	Measured concentration, mg/L	% Recovery	Measured concentration, mg/L	% Recovery
0.01	0.011	105.000	nd	nd	nd	nd
0.02	0.022	107.500	0.021	104.500	nd	nd
0.05	0.055	110.000	0.048	96.000	nd	nd
0.06	0.064	106.670	0.057	94.170	nd	nd
0.09	0.091	100.560	0.082	91.110	nd	nd
0.1	0.093	92.500	0.113	113.000	nd	nd
0.2	0.204	102.000	0.185	92.500	nd	nd
0.5	0.515	103.000	0.478	95.500	nd	nd
1	0.980	98.000	0.945	94.500	nd	nd
2	2.040	102.000	1.865	93.250	nd	nd
5	5.070	101.400	4.580	91.600	4.905	nd
8	8.025	100.310	7.865	98.310	7.935	97.960
10	9.855	98.550	10.660	106.600	9.920	97.250

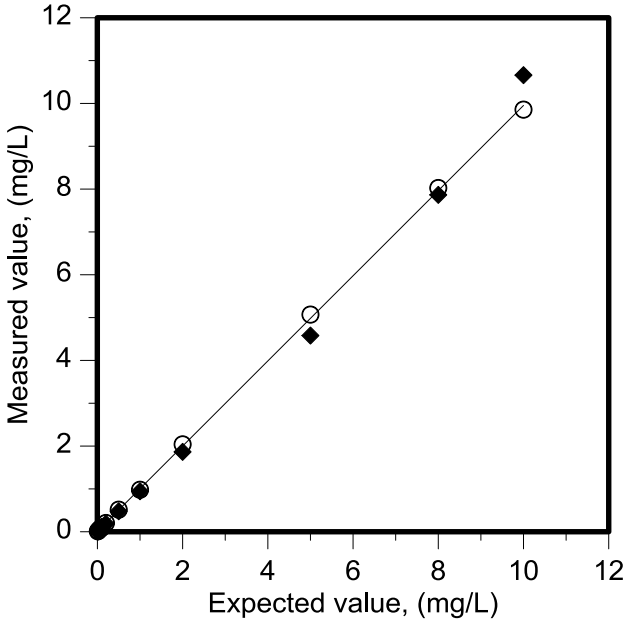


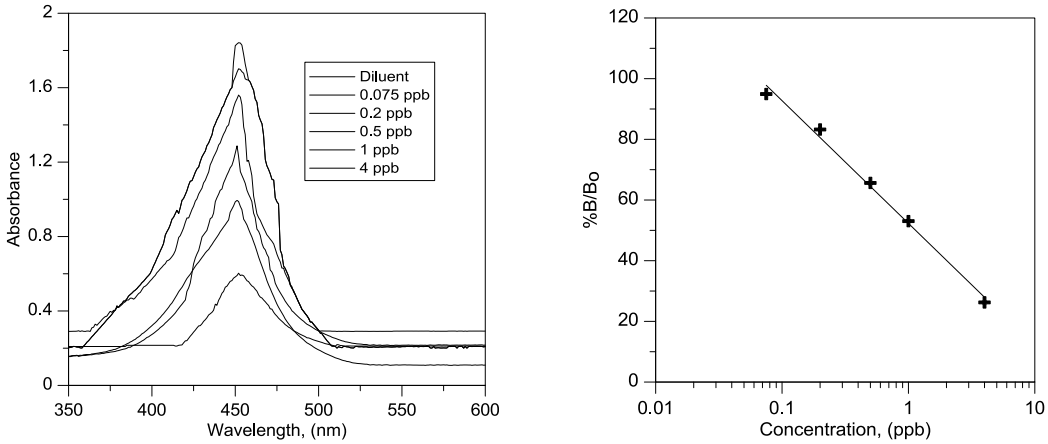
Fig. 3. Comparison of ELISA and Fluorometer results

ELISA method

As mentioned, an enzyme-linked immunosorbent assay (ELISA) method was used as a reference method for the analysis of glyphosate in waters. A Glyphosate ELISA Plate Kit was purchased from Abraxis LLC (Warminster, PA) and vortexing was performed using a Vortex Genie 2 (VWR international). The ELISA plate consisted of 96 test wells for each plate and analysis was performed with a UV-visible spectrophotometer (CARRY 50) equipped with a 0.1 cm quartz cell. All reagents and samples (water and soil) were prepared according to the guideline provided with the kit [53]. The analysis procedure was performed in accordance with the kit operating manual [53].

Results

The mean absorbance value for each of the standards was obtained from the UV-visible spectrum. The percentage ratio of the mean absorbance value of each standard (B) to the mean absorbance value of the diluent (B_0) was calculated and plotted against the concentration of glyphosate to obtain the five-point calibration curve (Fig.4.). This curve was then used to calculate the concentration of different samples (soil and water).

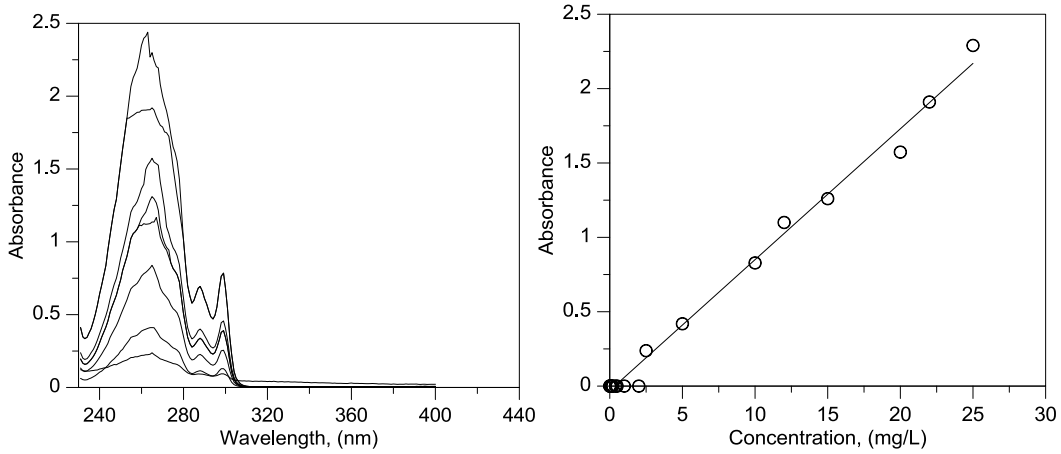


(a) **(b)**

Fig. 4. (a) Absorption spectra of glyphosate by ELISA method at different concentrations, and (b) constructed calibration curve.

Spectrophotometric method

A recent established direct spectrophotometric method [34] was also used in conjunction with the ELISA method for comparison with the fluorometric results. The fluorometric method shows higher precision compared to the spectrophotometric method for samples of lower concentration range (0.01 mg L^{-1} to 25 mg L^{-1}). For glyphosate sorption and desorption, the spectrophometric and fluorometric methods were of comparable precision.



(a) **(b)**

Fig. 5. (a) UV-visible absorbance spectra of derivatised glyphosate at different concentrations, and (b) constructed calibration curve.

CASE STUDY
Area of study

For the water and soil sampling, a family-operated farm was selected in the agricultural district of Parkes, in the central west of NSW (Fig.6). All investigations were conducted in accordance with the consent and ethics approval procedures of the University of NSW. The farmer and family have been using this land for the last five years for continuous cropping rotation with wheat and GM canola utilising no-till and control traffic management practices. They had planted over 1500 ha of GM canola in the last five years, and about 410 ha of GM canola in the last year. The waste associated with the application of herbicides, pesticides and fertilisers during the land preparation and overall growing season. The region is characterised by very slight undulations cut by shallow creeks. Characterisation studies found that 0.93-1.1% of organic matter is present in surface soils, with a pH of 4.5-5.5.

Sowing event, applications and precipitations

Fig. 7 shows the sowing event and the herbicide applications as well as daily rainfall during the studied period. Sowing of GM canola was conducted on 27/04/12; glyphosate application was performed three times before sowing the canola plant and another two times during the overall crop rotation. The herbicide application and sowing dates provided here were obtained from discussions with the farmer. Precipitation data recorded during the period were collected from the Bureau of Meteorology.

Sample collection

Water sampling

Water samples were collected in different episodes to allow for glyphosate applications and rainfall events. Three sampling locations were used: location 1 (L1), adjacent to the GM canola cultivation area; location 2 (L2), the drainage lines formed on the paddock; and location 3 (L3), at the end of the paddock where a wetland was formed by the water streams flowing through the cultivated area. Surface water samples were collected from two creeks using 50 mL clear glass vials (COSPAK) a few centimetres below the water surface, filling the container approximately half-full, and packed in an ice box. Sorption to glass did not appear to be an issue as there is no preference between collection in either glass or plastic [54] for glyphosate suggested in the literature. Samples were stored frozen in the dark in order to inhibit degradation [55] .

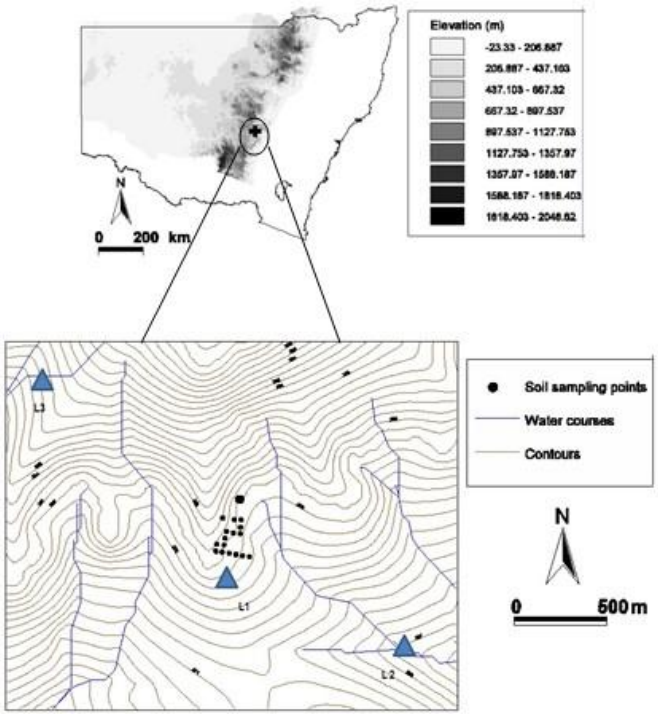
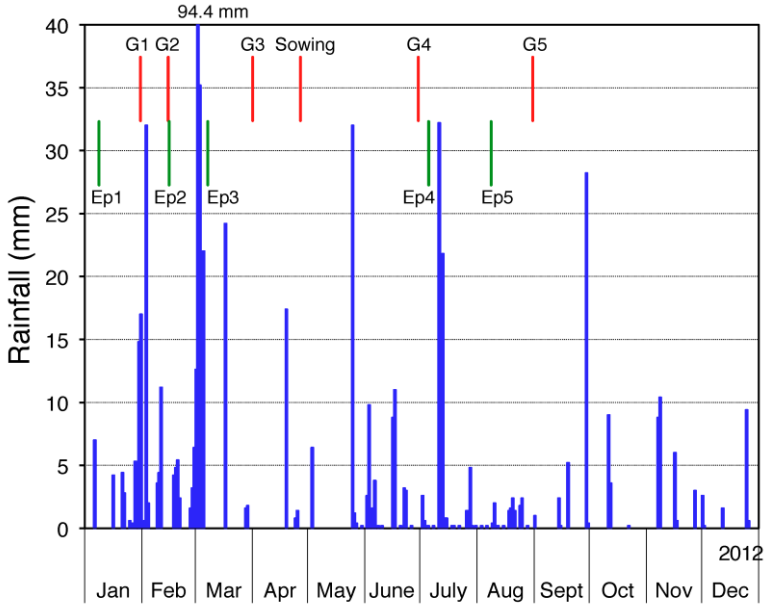


Fig. 6. Map of the region of GM canola cultivated area



Glyphosate Application	Approximate rate, (L/ha)
G1	1.2
G2	1.5
G3	1.2
G4	1.0
G5	1.2

Fig. 7. Precipitation, sowing events and herbicide applications in the field (G1 to G5) and timing of sampling events (Episodes 1 to 4).

Soil sampling

A stratified sampling pattern was chosen for soil sampling as the site is very large and complexities arise for other sampling patterns. The sampling density was determined by the NEPM sampling schedule. Samples were collected from different locations at different times of the year (Episodes 1 to 5 in Fig. 6) to allow for glyphosate applications and rainfall events. All samples were collected from sampling depths of up to 30 cm, using the NEPM sampling protocol, into glass containers, chilled and analysed for glyphosate within two weeks.

Separate soil samples were also collected and analysed for major soil properties including texture, major cations and other constituents, exchangeable ions, total Fe and total Al.

(a) (b) (c)

Fig. 8. Sampling details for soil samples (a) point near canola crop. (b) collecting surface soil (c) sampling up to 30 cm

Table 2: Identification of samples taken from the study area

Episode	Description	Month	Description
Episode 1	Before crop rotation	During January	Water sample from L1, L2, L3, Creek 1 and Creek 2. Soil sampling from different points in the paddock, Soil 1.
Episode 2	After second application of glyphosate	End of February	Water sample from L1, L2, L3, Creek 1 and Creek 2 Soil sampling from different points in the paddock, Soil 2.
Episode 3	After the rainfall	Beginning of March	Water sample from L1, L2, L3, Creek 1 and Creek 2 Soil sampling from different points in the paddock, Soil 3.
Episode 4	After fourth application of glyphosate	Middle of July	Water sample from L1, L2, L3, Creek 1 and Creek 2 Soil sampling from different points in the paddock, Soil 4.
Episode 5	After the rainfall	Beginning of August	Water sample from L1, L2, L3, Creek 1 and Creek 2 Soil sampling from different points in the paddock, Soil 4.

Results

The results of soil characterisation studies are shown in Table 3. The glyphosate analysis results of water and soil samples, showing the ELISA and direct fluorometric methods separately, are provided in Figs. 9 to 12.

Table 3. Major soil properties and composition (from 3 samples).

Properties		Value
Soil texture	Sand (%)	41.2 - 47.2
	Silt (%)	39.00 - 43.00
	Clay (%)	13.8 - 15.8
Metal content	SiO_2(%)	53.799-78.333
	Fe_2O_3(%)	5.633-17.597
	Al_2O_3(%)	9.018-14.271
	CaO(%)	0.253-9.21
	K_2O(%)	1.808-3.554
	TiO_2(%)	1.794-2.133
	SO_3(%)	0.833-0.955
	MnO(%)	0.232-0.277
	MoO_3(%)	0.022-0.033
	ZnO	0.030-0.037
	Rb_2O	0.031-0.039

Properties	Value
Total Solids (%)	86.9 - 98.4
P-Colwell (mg/kg)	20-140
pH ($CaCl_2$)	4.15 - 5.61
Conductivity (µs/cm)	12.9 - 114.50
Organic matter (%)	0.96 -1.5
Moisture Content (%)	4.37 - 19.94
CEC (mEq/100g)	3.8 - 7.5
Ex-Mg (mEq/100g)	0.36 - 1.6
Ex-K (mEq/100g)	0.97 -1.6
Ex-Na (mEq/100g)	0.04-0.18
Ex-Al (mEq/100g)	0.06-0.25
Ex-Ca (mEq/100g)	1.4-4
Aluminium (mg/kg)	11600-16500
Iron (mg/kg)	18100- 21500

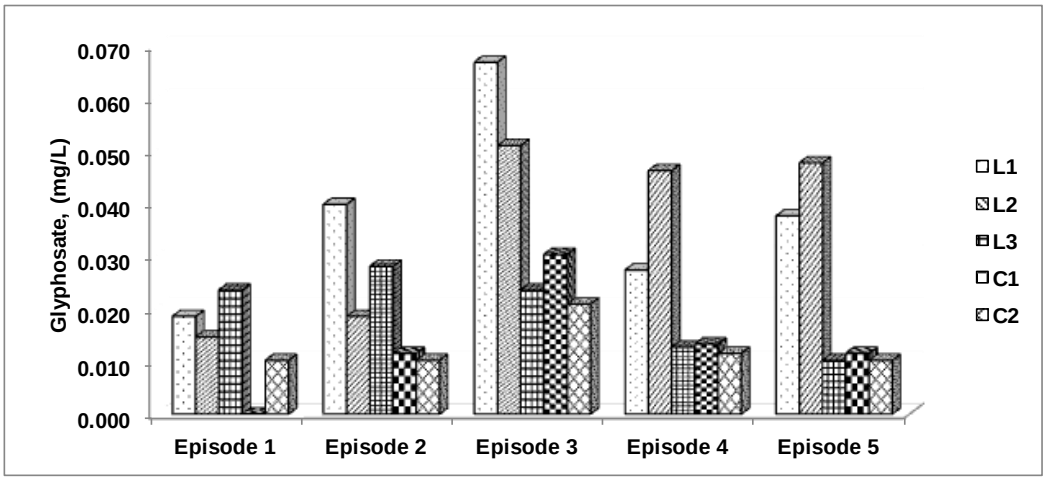


Fig. 9. Glyphosate concentration in water samples from different locations by ELISA method

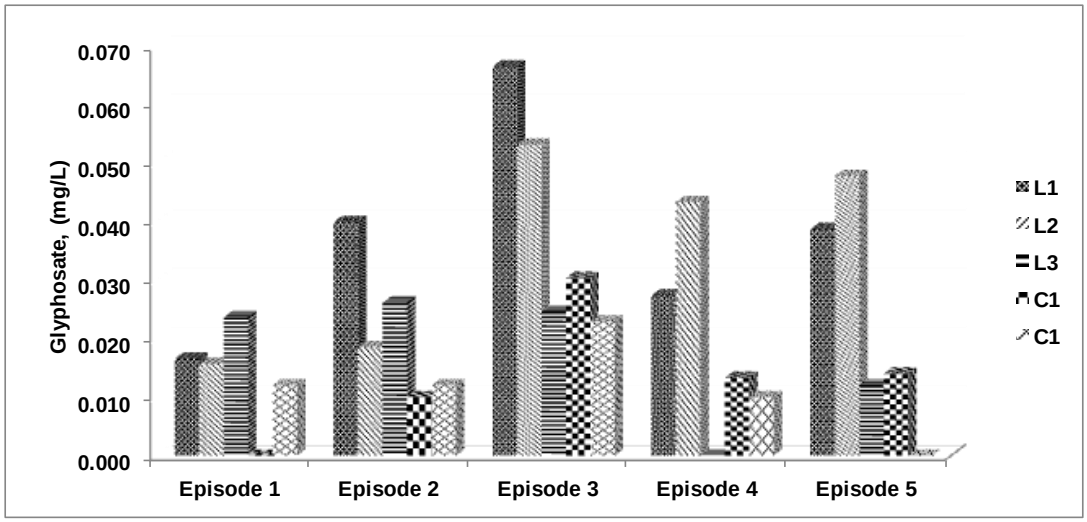


Fig. 10. Glyphosate concentration in water samples from different locations by direct fluorometric method

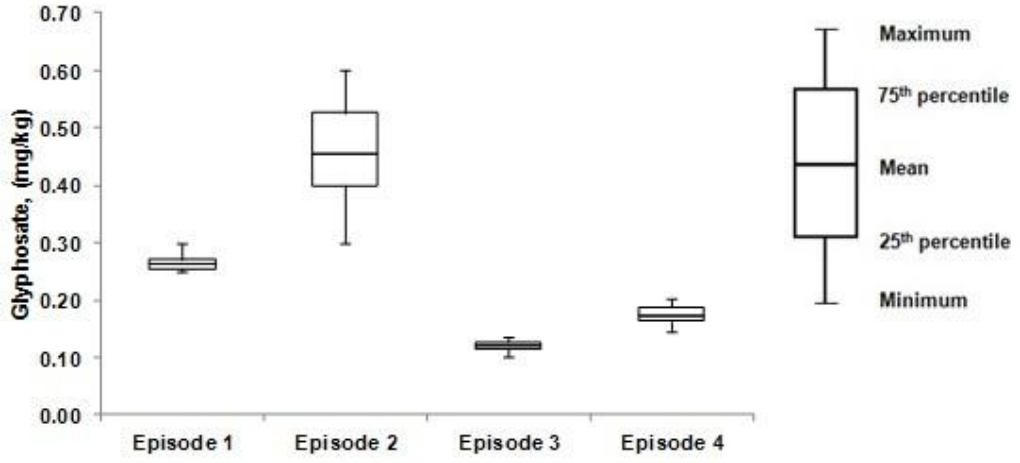


Fig. 11. Glyphosate concentration in soil samples from different locations by ELISA method

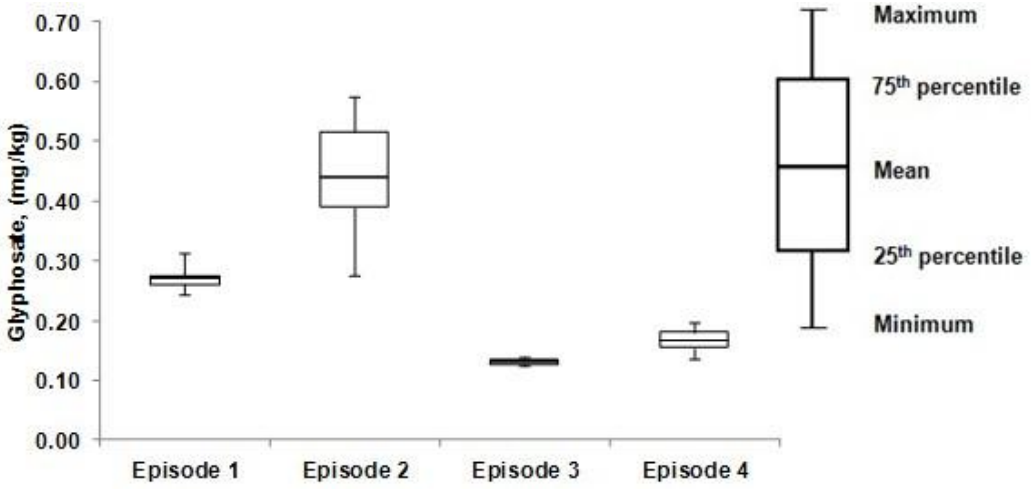


Fig. 12. Glyphosate concentration in soil samples from different locations determined by direct fluorometric method.

SOIL SORPTION ISOTHERMS

Glyphosate sorption to soil is usually described by the sorption isotherm which is a graph of the equilibrium surface excess or amount of a compound adsorbed, designated by C_s, plotted against the equilibrium solution concentration of the compound, designated by C_{eq}, at fixed temperature, pressure, and solution chemistry(e.g., pH and ionic strength [56].

Procedure:

The sorption isotherms were performed on three different soils from three different GM canola paddocks. The properties of these different soils were characterised and given in Table 4.

Table 4: Properties of the three different soils from GM canola paddock

Soil	pH	OC	CEC	Fe (%)	Al (%)	Sand (%)	Silt (%)	Clay (%)
A	4.5	0.94	6.81	5.633	9.018	47.2	39	13.8
B	4.8	1.1	7.4	17.597	14.271	43.2	29	27.8
C	5	0.9	4.75	7.649	11.03	41.2	43	15.8

Sorption isotherms were obtained by weighing 2.0 g of air dried samples of each soil into a number of 50 mL polypropylene centrifuge tubes. To each tube, 25 mL of 0.1 M KCl was added as a background electrolyte, and appropriate aliquots of standard solutions of glyphosate were added to cover the concentration range 5 ppm, 10 ppm, 12 ppm,15 ppm,20 ppm,22 ppm and 25 ppm. Small amounts of either KOH or HCl solutions were added to reach a constant pH of 4.5, 4.8 and 5 for the soils A, B and C. The tubes were shaken overnight with a mechanical shaker to achieve equilibration. The tubes were then centrifuged at 4000 rpm for 17 min, and the supernatants were withdrawn for derivatisation and further analysis. The sorbed amount (C_s) was calculated from the difference between the initial glyphosate concentration (C_i) and the concentration remaining in the supernatant solution (C_e).

Results

The sorption isotherms obtained by the analysis of glyphosate by both fluorometric and spectrophotometric method are shown in Fig 13, based on the calibration curves respectively in Fig 2. and Fig 5. The data were then fitted with both a Freundlich isotherm equation:

$$C_S = K_f C_{eq}^{1/n}$$

where K_f is a constant which indicates the relative sorption capacity of the adsorbent ($mg^{1-(1/n)}\ L^{1/n}$) /kg), *n* is a

constant, as well as a Langmuir linearized equation:

$$C_S = \frac{q_m b C_{eq}}{1 + b C_{eq}}$$

from which:

$$\frac{1}{C_S} = \frac{1}{q_m b}\frac{1}{C_{eq}} + \frac{1}{q_m}$$

where q_m is the maximum sorption capacity (mg/kg) and b is a constant related to the free energy of sorption (L/ mg). The results are listed in Table 5, and the Freundlich isotherms are shown as fit curves in Fig. 13.

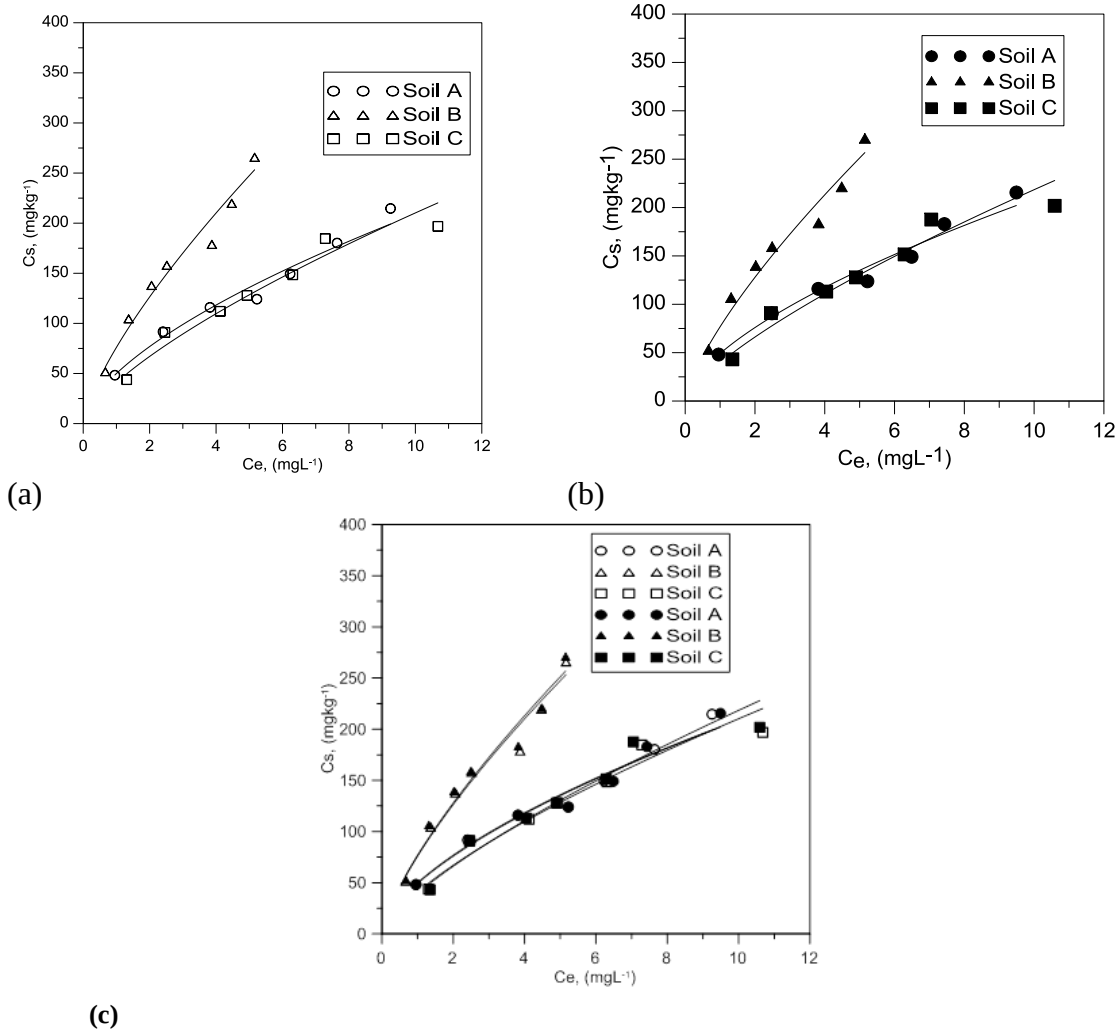


Fig.13. Sorption isotherms of glyphosate on different soils (a) spectrophotometric results (b) fluorometric results (c) comparison of spectrophotometric and fluorometric results. Solid lines indicate fitted Freundlich isotherms.

Table 5. Freundlich and Langmuir constants and coefficients of determination (R^2) for glyphosate sorption.

Methods	Soil	Freundlich Model			Langmuir Model		
		K_f	n	R^2	b	q_m	R^2
Spectrophotometric	A	49.967	1.61	0.9824	0.247	250	0.9849
	B	76.037	1.36	0.9743	0.167	526	0.9925
	C	40.936	1.41	0.9601	0.084	454	0.98
Fluorometric	A	49.11	1.59	0.9821	0.244	250	0.9833
	B	76.723	1.36	0.9714	0.147	588	0.9889
	C	39.53	1.35	0.9558	0.065	555	0.9754

Desorption

The samples of soil containing sorbed glyphosate were then subjected to desorption by using 0.1M KH_2PO_4 agitation (17 min), centrifugation (4000 rpm; 10 min) and filtration trough 0.45 µm filter paper. The extraction was repeated twice on solid residue, obtaining a 25 ml extract from each sample. Extracts were filtered through 0.45 µm filter. The derivatisation was then carried out using FMOC-Cl with further measurement by spectrophotometer and fluorometer. The results are listed in Table 6, and are also illustrated in Fig. 14.

Table 6. Glyphosate desorption by the treatment with 0.1 M KH_2PO_4

Glyphosate added, mg/L	Percentage desorption (Spectrophotometric method)			Percentage desorption (Fluorometric method)		
	Soil A	Soil B	Soil C	Soil A	Soil B	Soil C
5	50.893	32.687	35.082	50.717	32.622	35.314
10	32.445	36.401	25.908	32.434	36.963	24.511
12	27.633	35.938	30.000	28.020	36.058	29.788
15	42.940	39.818	38.652	43.010	39.424	37.340
20	39.615	43.695	42.014	40.157	43.285	41.357
22	45.774	39.673	46.537	44.936	39.879	45.518
25	41.098	34.270	44.818	40.924	34.022	43.904

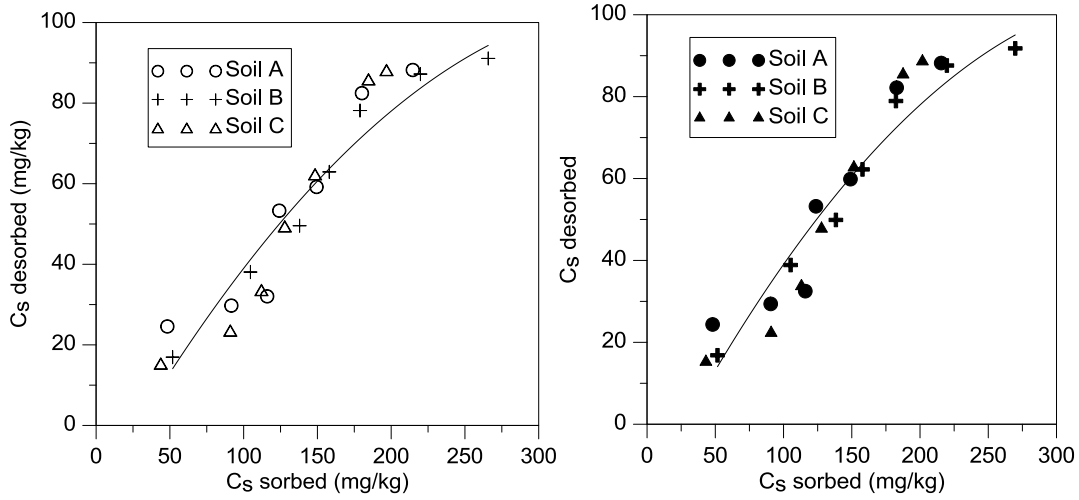


Fig.14. Desorption results of glyphosate from different soils (a) spectrophometric results (b) fluorometric results

DISCUSSION

The simple, fast and low cost fluorometric method developed herein may allow others in future to conduct broad-scale monitoring programs to assess whether levels of herbicides such as glyphosate are increasing in soils and surface waters, due to the increased cultivation of genetically modified crops over the last few years in Australia. For example, for a sampling program involving the collection of 100 samples over the course of the growing season and analysis for glyphosate, use of chomatographic methods would cost about $15000, whereas analysis using the direct fluorometric method would cost approximately $1000. This method also provides a cost-effective alternative for improving temporal and spatial monitoring, which would probably allow greater flexibility for monitoring agencies to develop broad scale projects for determining the environmental fate and transport of glyphosate.

As seen from the Figs 9 to 12, glyphosate concentrations in most of samples were detected by the ELISA method and fluorometric method. Some of the water samples, with glyphosate concentrations lower than 0.01 µg/mL, were not detectable by the fluorometric method. The water samples collected after the rainfall event in March show the highest concentrations of glyphosate. This suggests the washing out of freshly applied glyphosate by the highly rainfall events of early March. Glyphosate concentrations in water samples collected after the fourth application was not so pronounced, probably due to its lower dosage and also to the lower intensity of preceding rainfall events. Clearly, rainfall events play a notable role, transporting newly applied glyphosate into surface waters through the mechanisms of dilution or drift of the surface material by runoff.

For soil samples, a higher concentration was observed in the samples taken after second application of glyphosate (1.2 L/ha+1.5L/ha). This appears to reflect the applied doses in each case, as well as lower rainfall events.sThe samples collected after the rainfall event in March showed a decline in glyphosate concentration. It is also pointed out that the samples collected before the crop rotation showed some base level glyphosate concentration which could be related to the higher dose of herbicide applications conducted before crop rotation and sowing started, which were usually done to wash the herbicide and prepare the land for cultivation.

Results of glyphosate sorption measured by spectrophometric and fluorometric determination on the three soils at the different concentrations showed that the sorbed herbicide decreased in the order B>A>C for both cases. From Table 4, the order of sorption is approximately parallel to the content of iron and aluminium present in the soils, suggesting strong sorption of glyphosate through a ligand exchange mechanism. Based on soil concentrations C_s in mg/kg and aqueous concentrations C_e in mg/L, Freundlich isotherms $C_s = K_f C_e^{1/n}$, fitted the data well, with (diminishing) exponents n = 1.35–1.61 and coefficients K_f = 39.53–76.723 ($(mg^{1-(1/n)} L^{1/n})$ /kg)) (R^2 0.9588–0.9824). In case of Langmuir isotherm the values for the maximum sorption constants b and q_m ranged between 0.065-0.247 and 250-588 respectively.

The glyphosate desorption results for the three soils after 0.1 M KH_2PO_4 treatment indicates 24.5% to 50.8% of soil glyphosate is extractable by 0.1M KH_2PO_4 (Table 6 and Fig.14).

ENVIRONMENTAL IMPACT

Glyphosate concentrations in waters ranged from 0.010 to 0.067 mg/L and in the soil samples 0.1 mg/kg to 0.575 mg/kg. In accordance with the standard value of glyphosate concentration (1 mg/L) provided by the Australian Drinking Water Guideline [23] this study reveals glyphosate levels within the human health based limits. However, they approach the Canadian Water Quality Guidelines for the Protection of Aquatic Life, of 0.065 mg/L. The data therefore suggest the need for widespread monitoring of glyphosate, especially in the areas of genetically modified crops cultivation, to better assess its human health and environmental impacts.

An increase in broad-scale glyphosate monitoring is also suggested by recent developments in Australian and state government policies to encourage the use of biofuels. In Australia, biofuel production increased from 2009–10 to 2010–11, continuing the trend of recent years [57]. Australian biofuel production reached a total of 419 mega litres (ML) in 2010–11; this was an increase from 354 ML in 2009–10. Ethanol production increased from 269 ML to 319 ML and biodiesel production increased from 85 ML to 100 ML [58]. In Canada, the federal government projected ethanol production growing from 42 million litres in 2006 to approximately 799 million litres by 2010 [59]. In United States the target production of 35 billion gallons of renewable fuel by 2017 has led to an increase in ethanol production [60] and in 2006 ethanol was accounted for 3.5% of the total U.S. fuel consumption. About 98% of the all U.S. ethanol production is corn based which is gradually accomplished by converting the existing cropland to corn and willingly planting corn in consecutive growing seasons rather than following a different crop rotation plan. Furthermore the farmers are relying on different pesticides and

genetically modified varieties of corn for protecting their crops against large scale crop failure, which are closely related to RoundUp Ready crops

CONCLUSIONS

A simple, fast and low cost fluorometric method has been developed for the determination of glyphosate herbicide in environmental and biological samples which was also used to perform sorption isotherms on different soils from Parkes, NSW. Glyphosate was satisfactorily determined with the lower detection limit of 0.01 µg/mL. The investigated method was applied successfully to environmental samples, with recovery values of 91.11 to 113% and 92.50 to 110% for the fluorometric and ELISA methods respectively.

A case study investigation was conducted of farmland from the Parkes region of New South Wales, Australia, cultivated with genetically modified canola, involving the determination of glyphosate (N-(phosphonomethyl)glycine) concentrations in water and soils, and its sorption. The soils are classified as loam under the USDA system (clay 13.8-15.8%, silt 39-43%, sand 41.2-47.2%). Soil and water samples were then collected using the NEPM sampling protocol into glass containers, chilled and analysed within two weeks. The samples were collected in multiple episodes, taking account of glyphosate and pesticide crop applications. The soil and water physical and chemical properties were characterised, and glyphosate levels were analysed by fluorometric and ELISA methods. Field concentrations of glyphosate in water ranged between 0.01 - 0.067 mg/L and in soil between 0.10 - 0.575 mg/kg. The aqueous levels lie below Australian and international drinking water guidelines, but reach a Canadian freshwater guideline. Glyphosate levels varied with time of application and rainfall events. Glyphosate sorption isotherms were also constructed by batch tests on several soils, and were fitted with Freundlich and Langmuir isotherms. Desorption tests indicated 25% to 58% of soil glyphosate is extractable by 0.1M KH_2PO_4.

Work is now underway on leachate trials of glyphosate in soils from the case study site, including the effects of dissolved phosphorus, pH and background electrolyte.

ACKNOWLEDGEMENTS
The authors wish to thank the members of Water Quality Lab of UNSW Canberra for their technical support.

REFERENCES

1. Genetically Modified Crop fact sheet, Depatment of Agriculture, Fisheries and Forestry, Australian Government.
2. GM Canola 2010 - Progress, New South Wales,Victoria and WesternAustralia,Viewed:25-09-2012. Available from *http://www.afaa.com.au/n_gmcanola2010_progress.asp*.
3. Monsanto Australia Limited, Ripper Roundup Ready ®Results: GM Canola a 2008 success, *http://www.afaa.com.au/GM_canola_2008/RIPPER_ROUNDUP_RESULTS_MEDIA_RELEASE_Final.pdf*
4. Hartzler, B., et al., Understanding Glyphosate To Increase Performance, in The Glyphosate, Weeds, and Crops Series. 2006.
5. Piccolo, A., et al., Adsorption and desorption of glyphosate in some European soils. Journal of Environmental Science and Health, 1994. **B29**(6): p. 1105-1115.
6. DeJonge, H. and L.W. DeJonge, Influence of pH and solution Composition on the Sorption of Glyphosate and Prochloraz to a Sandy Loam soil. Cheraosphere, 1999. **39**(5): p. 753-763.
7. Gimsing, A.L. and O.K. Borggaard, Effect of Kcl and CaCl2 as Background Electrolytes on the Competitive Adsorption of Glyphosate and Phosphate on Geothite. Clays and Clay Minerals., 2001. **49**(3): p. 270–275.
8. Sari Autio, et al., Adsorption of sugar beet herbicides to Finnish soils. Chemosphere 2004. **55**: p. 215–226.
9. A.L.Gimsing, O.K.Borggaard, and M. Bang, Influence of Soil Composition on Adsorption of Glyphosate and Phosphate by Contrasting Danish Surface Soils. European Journal of Soil Science, 2004. **55**: p. 183–191.
10. Gimising, A.L. and O.K.Borggaard, Competitive Adsorption and Desorption of Glyphosate and Phosphate on Clay Silicates and Oxides. Clay Minerals 2002. **37**: p. 509–515.
11. A.L. Gimsing, C. Szilas, and O.K. Borggaard, Sorption of glyphosate and phosphate by variable-charge tropical soils from Tanzania. Geoderma, 2007. **138** (1–2): p. 127–132.
12. Attaallah, M.A., The Kinetic Study of Glyphosate Leachate in Palestinian Soil at Different Concentrations, in An-Najah National University Faculty of Graduate Studies. 2011. p. 13-14.

13. Sprankle, P., W.F. Meggitt, and D. Penner, Adsorption, Mobility, and Microbial Degradation of Glyphosate in the Soill. Weed Science, 1975. **23**(3).
14. Gerritse, R.G., J. Beltran, and F. Hernandeg, Adsorption of atrazine, simazine, and glyphosate in soils of the Gnangara Mound, Western Australia. Australian Journal of Soil Research, 1996. **34**(4): p. 599-607.
15. Yu-jun, , W., Z. Dong-mei, , and S. Rui-juan, Effects of phosphate on the adsorption of glyphosate on three different types of Chinese soils. Journal of Environmental Sciences 2005. **17**(5): p. 711-715.
16. Miles, C.J. and H.A. Moye, Extraction of Glyphosate Herbicide from Soil and Clay Minerals and Determination of Residues in Soils. J. Agric. Food Chem, 1988. **1088**(36): p. 488-491
17. Glass, R.L., Adsorption of Glyphosate by Soils and Clay Minerals. J. Agrlc. Food Chem. , 1987. **35**(4): p. 497-500
18. Morillo, E., et al., Glyphosate adsorption on soils of di erent characteristics. Infuence of copper addition. Chemosphere 2000. **40** p. 103-107.
19. Sheals, J., S. Sjoberg, and P. Persson, Adsorption of Glyphosate on Goethite: Molecular Characterization of Surface Complexes. Environ. Sci. Technol., 2002. **36**: p. 3090-3095.
20. Barja, B.C. and M.D.S. Afonso, Aminomethylphosphonic Acid and Glyphosate Adsorption onto Goethite: A Comparative Study. Environ. Sci. Technol., 2005. **39**: p. 585-592.
21. WHO Guidelines for Drinking-water Quality, Glyphosate and AMPA in Drinking-water, WHO/SDE/WSH/03.04/97, updated on June 2005.
22. Foreign funds to fertilise 'dining boom', SUE NEALES RURAL REPORTER, in The Australian. 18 July 2012.
23. National Water Quality Management Strategy, Australian Drinking Water Guidelines -6, Vol 1, 2011
24. WHO guidelines for drinking-water Quality, Vol 1. 2008.
25. Drinking water contaminants, Environmental Protection Agency (EPA), USA, Viewed: 16 April 2013;. Available from: http://water.epa.gov/.
26. U.S. EPA Office of Ground Water and Drinking Water. (2002) Technical Fact Sheet on Glyphosate. *http://www.epa.gov/safewater/pdfs/factsheets/soc/glyphosa.html*. May 22, 2002 update.
27. STANDARDS, O.D.W.Q., Safe Drinking Water Act. 2002. **169**(03).
28. Canadian Water Quality Guidelines for the Protection of Aquatic Life, updated 7.1, 2007
29. Surface water quality guidelines for use in Alberta, Environmental Assurance Division, 1999
30. National Drinking Water Database. Available from *http://www.ewg.org/tap-water/chemical-contaminants/?file=contaminant&contamcode=2034*.
31. Heong, L.L.L.a.C.Y. Danish water contaminated by RoundUp, Ban imposed , Third world network biosafety information service. 2003; Available from: http://www.twnside.org.sg/title/service76.htm.
32. Jonathan D. Byer, et al., Low Cost Monitoring of Glyphosate in Surface Waters Using the ELISA Method: An Evaluation. Environ. Sci. Technol., 2008. **42**: p. 6052–6057.
33. Birchip Cropping Group GM canola grower case studiesI, BCG/GRDC project – BWD00016.
34. Carolina V. Waiman, et al., A simple and rapid spectrophotometric method to quantify the herbicide glyphosate in aqueous media. Application to adsorption isotherms on soils and goethite. Geoderma, 2012. **170**: p. 154-158.
35. Engelhaupt, E., Perspective: Biofueling Water Problems. Environ Sci Technol, 2007. **41**(22): p. 7593-7595.
36. Shuji Kodama, et al., A Fast and Simple Analysis of Glyphosate in Tea Beverages by Capillary Electrophoresis with On-Line Copper(II)-Glyphosate Complex Formation. Journal of Health Science, 2008. **54**(5): p. 602-606.
37. Kun Qian, et al., Residue determination of glyphosate in environmental water samples with high-performance liquid chromatography and UV detection after derivatization with 4-chloro-3,5-dinitrobenzotrifluoride. Analytica Chimica Acta, 2009. **635**(2): p. 222-226.
38. Francisco Sánchez-Bayoa, Ross V. Hyneb, and K.L. Desseilleb, An amperometric method for the detection of amitrole, glyphosate and its aminomethyl-phosphonic acid metabolite in environmental waters using passive samplers. Analytica Chimica Acta, 2010. **675**(2): p. 125–131.
39. Everlyne A. Songa, et al., Electrochemical detection of glyphosate herbicide using horseradish peroxidase immobilized on sulfonated polymer matrix. Bioelectrochemistry, 2009. **75** (2): p. 117-123.
40. Constantine D. Stalikas and C.N. Konidari, Analytical methods to determine phosphonic and amino acid group-containing pesticides. Journal of Chromatography A, 2001. **907**(1-2): p. 1-19.
41. Hiroyuki Kataoka, et al., Simple and rapid determination of the herbicides glyphosate and glufosinate in river water, soil and carrot samples by gas chromatography with flame photometric detection. Journal of Chromatography A, 1996. **726**(1-2): p. 253-258.
42. Su-Hsiang Tseng , et al., Simultaneous Quantification of Glyphosate, Glufosinate, and Their Major

Metabolites in Rice and Soybean Sprouts by Gas Chromatography with Pulsed Flame Photometric Detector. J. Agric. Food Chem 2004. **52**(13): p. 4057–4063.
43. Maxim V. Khrolenko and P.P. Wieczorek, Determination of glyphosate and its metabolite aminomethylphosphonic acid in fruit juices using supported-liquid membrane preconcentration method with high-performance liquid chromatography and UV detection after derivatization with p-toluenesulphonyl chloride. Journal of Chromatography A, 2005. **1093**(1-2): p. 111-117.
44. Isabel Durán Merás, Teresa Galeano Díaz, and M.a.A. Franco, Simultaneous fluorimetric determination of glyphosate and its metabolite, aminomethylphosphonic acid, in water, previous derivatization with NBD-Cl and by partial least squares calibration (PLS). Talanta, 2005. **65**(1): p. 7-14.
45. Jennifer S. Ridlen, Gary J. Klopf, and Timothy A. Nieman, Determination of glyphosate and related compounds using HPLC with tris(2,2′-bipyridyl)ruthenium(II) electrogenerated chemiluminescence detection. Analytica Chimica Acta, 1997. **341**(2-3): p. 195-204.
46. María Ibáñez, et al., Residue determination of glyphosate, glufosinate and aminomethylphosphonic acid in water and soil samples by liquid chromatography coupled to electrospray tandem mass spectrometry. Journal of Chromatography A, 2005. **1081**(2): p. 145-155.
47. R.J. Vreeken , et al., Selective analysis of the herbicides glyphosate and aminomethylphosphonic acid in water by on-line solid-phase extraction–high-performan liquid chromatography–electrospray ionization mass spectrometry. Journal of Chromatography A, 1998. **794**: p. 187–199.
48. Albers, C.N., et al., The influence of organic matter on sorption and fate of glyphosate in soil – Comparing different soils and humic substances. Environmental Pollution, 2009. **157**(10): p. 2865-2870.
49. Glass, R.L., Colorimetric determination of glyphosate in water after oxidation to orthophosphate. Anal. Chem., , 1981. **53**(6): p. 921-923.
50. M. Rasul Jan, et al., Glyphosate herbicide residue determination in samples of environmental importance using spectrophotometric method. Journal of Hazardous Materials, 2009. **169**(1-3): p. 742-745.
51. Hiroshi Miyano, et al., Influences of metal ions on the reaction of amino and imino acids with fluorogenic reagents. Analytical Biochemistry, 1985. **150**(1): p. 125-130.
52. Glass, R.L., Liquid Chromatographic Determination of Glyphosate in Fortified Soil and Water Samples. J. Agric. Food Chem, 1983. **31**: p. 280-282.
53. Abraxis Glyphosate Plate Assay Kit, 96T, PN 500086,Operating Manual; Abraxis LLC: Warminster, PA; *http://www.abraxiskits.com/*.
54. Humphries, D., G. Byrtus, and A.-M. Anderson, Glyphosate Residues In Alberta's Atmospheric Deposition, Soils And Surface Waters 2005.
55. Mallat, E. and D. Barceló, Analysis and degradation study of glyphosate and of aminomethylphosphonic acid in natural waters by means of polymeric and ion-exchange solid-phase extraction columns followed by ion chromatography–post-column derivatization with fluorescence detection. Journal of Chromatography A, 1998. **823**(1-2): p. 129-136.
56. Tan, K.H., Principles of Soil Chemistry. Vol. Third Edition. 1998: Taylor & Francis.
57. Commission, A.C.a.C., Monitoring of the Australian petroleum industry. 2011.
58. APAC biofuels consultants, Australian Biofuels 2011–12, September 2011, pp. 14–5.
59. Environmental Protection Act. Ontario Regulation 535/05: Ethanol in Gasoline, *http://www.e-laws.gov.on.ca/html/regs/english/elaws_regs_050535_e.htm*.
60. Division, U.S.A.C.o.E.G.L.O.R., The Potential Impacts of Increased Corn Production for Ethanol in the Great Lakes-St. Lawrence River Region. 2007.